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SYMPATHETIC VASOCONSTRICTION AND VASODILATATION IN THE SUBMAXILLARY GLAND

by

© MARCUS WILLIAM WARENYCIA

A THESIS
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Sympathetic Vasoconstriction and Vasodilatation in the Submaxillary Gland submitted by Marcus William Warenycia in partial fulfilment of the requirements for the degree of Master of Science

ABSTRACT

The vasoconstriction and vasodilatation resulting from sympathetic nerve stimulation have been studied in cat and rabbit submaxillary glands. Whereas the vasodilatation seen in the rabbit may be accounted for by reactive hyperemia alone, that observed in the cat appears to be more complex. Attempts to separate vasoconstriction and vasodilatation in the cat by judicious selection of stimulation parameters were unsuccessful. The effects of different voltages, frequencies, and pulse durations were similar on both vasoconstriction and vasodilatation; the threshold voltages for both responses were similar, if not identical. Vasodilatation never appeared during sympathetic nerve stimulation; simultaneous administration of α-adrenoceptor antagonists failed to affect the onset of vasodilatation. Administration of Bradykinin potentiating factor (BPF) to cats that had been subjected to parasympathetic decentralization of one submaxillary gland did not result in enhanced vasodilatation, thus excluding kinin formation as a possible mechanism underlying sympathetic vasodilatation. Electron microscopy of postganglionic sympathetic fibres innervating the submaxillary gland revealed a relatively homogeneous population of unmyelinated fibres. Size differences were sufficiently small to preclude separation of different fibres by electrical stimulation. Positive or negative duct pressures applied concomitantly with sympathetic nerve stimulation did not affect the subsequent vasodilatation, suggesting that the change in blood flow could not be attributed to mechanical effects within the gland. Although the mechanism underlying

sympathetic vasodilatation remains unclear, the close association of this response with electrical stimulation strongly suggests the existence of sympathetic vasodilator fibres which, upon stimulation, would release noradrenaline, thereby directly activating vascular β-adrenoceptors within the gland.

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TABLE OF CONTENTS

CHAPTER PAGE

I. HISTORICAL SURVEY AND LITERATURE REVIEW 1

Vasomotor Nerve Fibres 1

Metabolic Vascular Mediators 4

Microcirculation Considerations 7

Neurotransmitters of Vasomotion 8

α- and β-Adrenergic Receptors (Adrenoceptors) 10

The Role of Kallikrein in Mediating Vasodilatation in the Submaxillary Gland 13

Other Aspects 21

Summary and Objectives of Present Work 24

II. MATERIALS AND METHODS 26

Animals 26

Anaesthesia 26

Preparative Surgery 26

Nerve Stimulation 27

Blood Flow 29

Measurement of Duct Pressure 29

Recording 31

Denervation 31

Drugs 32

Electron Microscopy of Sympathetic Nerves 32

TABLE OF CONTENTS (Cont.)

CHAPTER PAGE

III. RESULTS 34

Vascular Response to Nerve Stimulation in the Submaxillary Gland of the Cat and Rabbit 34

The Relationship Between Stimulation Parameters Used for Sympathetic Nerve Stimulation and the Evoked Vascular Response in the Cat 38

Experiments Concerning the Existence of Specific Dilator Nerve Fibres in the Cat Submaxillary Gland 39

Does Kallikrein Play Any Role in the Sympathetic After-Dilatation? 49

Could the After-Dilatation Arise as a Hemodynamic Adjustment in the Microcirculation? . . 51

IV. DISCUSSION 55

****

REFERENCES 62

VITA 71

APPENDIX 68

LIST OF TABLES

Table	Description	Page
I	Different Stimulus Parameters Used in Sympathetic Nerve Stimulation	28
II	Pharmacological Agents Used in Experiments	33

Appendix

III	Summary of Experiments	68
IV	Quantitative Evaluation of the Vascular Response to Sympathetic Nerve Stimulation	69
V	Regression Analysis and Least Squares Plot	70

LIST OF FIGURES

Figure		Page
1.	Schematic Representation of the Experimental Set-up for Studying Blood Flow	30
2.	Blood Flow Changes in the Cat Submaxillary Gland Seen in Response to Parasympathetic and Sympathetic Stimulation	35
3.	Blood Flow Changes in the Rabbit Submaxillary Gland Seen in Response to Parasympathetic and Sympathetic Stimulation	36
4.	Enlargement of the Sympathetic Response Seen in Fig. 2	37
5.	Voltage-response Relationships for Paired Vasoconstrictions and Vasodilatations	41
6.	Postganglionic Fibres Running to the Submaxillary Gland of the Cat From the Superior Cervical Ganglion	44
7.	Histogram of Size Distribution of Postganglionic Unmyelinated Fibres of the Superior Cervical Ganglion of the Cat	45
8.	Effects of α-Adrenoceptor Blockade Following Administration of Dibozane	47
9.	Increased Sympathetic Response in the Submaxillary Gland of the Cat Following Section of the Chorda Tympani	50
10.	Sympathetic Response During Positive Duct Pressures	53

I. HISTORICAL SURVEY AND LITERATURE REVIEW

A. Vasomotor Nerve Fibres

The first significant investigation of the physiology of the submaxillary gland began in 1851, when Ludwig was able to demonstrate that electrical stimulation of the parasympathetic nerve, the chorda tympani, or of the cervical sympathetic nerve trunk, resulted in secretion of saliva from the cat's submaxillary gland.

In the same year that Ludwig discovered secretomotor nerve fibres, Claude Bernard made the discovery of vasomotor control by finding in the rabbit that the cervical sympathetic chain exerted a vasoconstrictor influence on the blood vessels of the ear. Extending his investigations to the submaxillary gland, Bernard found, in 1858, that electrical stimulation of the chorda tympani nerve resulted in a marked increase in the venous outflow from the gland, which he regarded as due to vasodilatation. On stimulating the cervical sympathetic nerves, in contrast, there was a marked decrease in venous outflow, or vasoconstriction.

From these observations, Bernard concluded that both vasodilator and vasoconstrictor nerve fibres existed. Furthermore, he regarded this system of dual neurovascular control, with its complementary components, as being responsible for the local regulation of blood flow through the submaxillary gland. He concluded that this was

an example of a homeostatic mechanism.

Heidenhain (1883), who studied both the secretory and vasomotor innervation of the cat's submaxillary gland, confirmed Bernard's results. In 1872, Heidenhain observed that the vasodilatation seen upon chorda stimulation was resistant to atropine blockade.

At the turn of the century, the existence of sympathetic vasoconstrictor fibres to the submaxillary glands was accepted. Langley (1900), the recognized authority on the sympathetic nervous system, wrote only of the vasoconstrictor action of the cervical sympathetic to the submaxillary gland of the cat. Similarly, Bayliss (1905), in a review on the subject of vasomotor innervation, also held this view.

In 1907, A.J. Carlson supplied the first evidence for the possible existence of vasodilator fibres in the sympathetic nerves running to the submaxillary gland of the cat. By stimulation of the cervical sympathetic trunk with an induction coil, Carlson demonstrated a significant increase in the venous outflow in addition to the previously noted vasoconstriction, or reduction in venous outflow. The magnitude of the sympathetic dilatation appeared not to be as great as that accompanying stimulation of the chorda tympani. There were also some features that established the sympathetic vasodilatation as a curious effect. Among Carlson's more interesting conclusions were the following:

1. In most cases stimulation of the sympathetic, either pre- or post-ganglionically, resulted in immediate vasodilatation. Occasionally, however, the dilatation

did not develop until after stimulation had been discontinued; no change, however, was observed in blood flow during stimulation.

2. Less frequently, the response to sympathetic stimulation was one of vasoconstriction, followed upon cessation of stimulation by vasodilatation.
3. It was possible to produce two sequential dilatations. Specifically, the onset of the first dilatation occurred during stimulation, followed by a return to basal flow but never below. Upon termination of stimulation, another dilatation much larger in magnitude than the first one could be seen.
4. Vasodilatation was sometimes seen without vasoconstriction or secretion by stimulating with weak, interrupted current.
5. It was possible to separate secretomotor and vasomotor effects with graded doses of atropine; a dose of atropine that abolished secretion left dilatation and constriction unaffected.
6. Atropine appeared to exert an effect on the superior cervical ganglion. At a stage of atropinization where secretion was blocked, preganglionic stimulation resulted in slight vasodilatation, whereas postganglionic stimulation produced marked vasoconstriction.

McLean (1908) confirmed these findings with the exception that only vasoconstriction or only vasodilatation could be obtained in response

to sympathetic stimulation. Adrenaline injected intravenously also produced either vasoconstriction or vasodilatation. Animals that responded with vasoconstriction to adrenaline also responded in this way to sympathetic stimulation; this type of correspondence was also seen to apply to the dilatation. McLean's results differed from those of the earlier work of Elliott (1905), who observed only vasoconstriction in response to either adrenaline or sympathetic nerve stimulation.

Carlson and McLean (1908) suggested that a relationship existed between blood flow and secretion in that vasoconstriction and the production of viscous saliva were related; conversely, vasodilatation was associated with the secretion of watery saliva. Thus, Carlson and McLean adopted the view that the reduction in blood supply caused by sympathetic vasoconstrictor fibres was responsible for the reduction in volume of secreted saliva seen on sympathetic stimulation. The augmented flow of saliva following the dilatation was attributed to the increase in blood flow mediated by sympathetic vasodilator fibres.

B. Metabolic Vascular Mediators

Barcroft (1908) was the first to challenge Carlson's assertion that true sympathetic vasodilator fibres existed. Using adrenaline to mimic the effects of sympathetic nerve stimulation, Barcroft demonstrated that it caused a long-lasting vasoconstriction, followed by an extensive vasodilatation. Furthermore, the dilatation reached its maximum after the secretion of saliva had reached a maximum. The dilatation and secretion produced by adrenaline as well as that

elicited by sympathetic nerve stimulation were both abolished by ergotoxine, which had been shown by Dale (1906) to block the effect of stimulation of sympathetic vasoconstrictor fibres. Barcroft concluded that the vasodilatation was caused by metabolites acting on the blood vessels of the submaxillary gland. These metabolites were assumed to be products of secretory metabolism.

In later work, Barcroft and Piper (1912) further elaborated on the effects of adrenaline. They concluded that the dilatation is not caused by adrenaline since it persisted after the systemic effects of adrenaline on the blood pressure had subsided. That the dilatation was maintained after secretion of saliva had stopped was taken as further evidence that the dilatation was a consequence of secretory activity. In addition, Barcroft and Piper found that the oxygen consumption of the gland increased following an injection of adrenaline but did not reach a maximum until a time corresponding to that of the maximal dilatation. Thus, it was concluded that metabolites, a consequence of secretory metabolism, were responsible for the sympathetic vasodilatation. Barcroft (1914) later modified his view by stating the vasodilatation was possibly initiated by dilator nerve fibres but maintained by metabolic products.

Besides studying the vasomotor response to sympathetic stimulation, Barcroft and Mueller (1912) had studied the effects of stimulation of the parasympathetic nerve, the chorda tympani. Examining the oxygen consumption of the active submaxillary gland before and after administration of atropine, they found that while the secretory response to chorda stimulation was blocked by atropine,

the metabolic activity (as indicated by an increase in oxygen consumption) and vasodilatation were unaffected. These findings led Barcroft and Mueller to conclude that this increased blood flow, caused by chorda lingual nerve stimulation, was also secondary to the increased metabolic activity of the secretory cells.

Bayliss (1923) criticized the analysis of Barcroft and Mueller by pointing out that there was no quantitative correlation between the increased metabolic activity and the vasodilatation elicited by chorda stimulation after secretory blockade by atropine. Bayliss reaffirmed his position that true vasodilator fibres existed in the chorda tympani, and that these fibres were responsible for parasympathetic vasodilatation in the submaxillary gland.

It is perhaps apparent by this time that two different schools of thought existed on the nature of the vasodilatation. One school maintained that the chorda vasodilatation was indeed caused by true dilator nerve fibres exerting a direct influence on the blood vessels of the submaxillary gland, an opinion shared by Dale and Gaddum (1930). To some extent this position was quite tenable. Chauchard and Chauchard (1929) had evidence which seemed to show that the secretory and dilator responses possessed different chronaxies. Later, Beznak (1934) was able to separate secretion and dilatation in the dog submaxillary gland with differential electrical stimulation; this certainly suggested the existence of separate fibres. These findings and others were taken as good evidence for the case of special parasympathetic vasodilator fibres. The same could not be said, however,

with equal conviction for the case of sympathetic vasodilator fibres. Therefore, the school of thought which maintained that metabolites were responsible for vasodilatations argued that the sympathetic vasodilatation was secondary to secretory metabolism. The fact that the sympathetic dilatation was unseparable from secretion was the strongest argument for this view. However, no attempt was made to even speculate on the nature of a vasoactive intermediary substance presumably formed in the process of secretion.

In 1936, Werle and von Roden discovered that a powerful vasodilator substance, kallikrein (the pharmacological effects of which were atropine resistant) was present in the submaxillary gland. As a result of their studies on the vasodilator properties of saliva, Ungar and Parrot (1936) were quick to suggest that kallikrein was indeed the chemical intermediary of the chorda vasodilatation. Their hypothesis was as follows: parasympathetic nerve stimulation in some way released kallikrein from glandular cells into the interstitial fluid; this kallikrein then acted upon glandular vessels to produce functional dilatation. Their hypothesis was to lie dormant for nearly two decades before attempts were made to obtain experimental proof.

C. Microcirculation Considerations

A completely different approach to studying the problem of vasodilatation in the submaxillary gland was adopted by Holzöhner and Niessing (1936a, b), who examined the microcirculation during parasympathetic vasodilatation in the presence of pilocarpine in the dog and the cat. By the use of subcapsular injection of India ink into

the lymph spaces and intra-arterial vital staining with indigo carmine, they supplied evidence which was interpreted to indicate that the increased blood flow during chorda stimulation was attributable to the opening up of arteriovenous anastomoses. Under this scheme, the flow of blood through the capillaries surrounding the ends of the glandular tubules would be greatly reduced owing to the diversion of blood proximally into the anastomoses. As a result of decreasing the blood supply in the microcirculation, the net effect would then be seen as an increase in venous outflow.

This hypothesis came under criticism from Brücke and Zwiauer (1938), who had two main objections. Firstly, it was pointed out that inadvertent stimulation of the digastric muscle as well as that of the chorda tympani nerve took place; the contraction of this muscle was held responsible for mechanical obstruction of capillary blood flow by the pressure exerted on duct passages within the gland. Secondly, the large doses of pilocarpine used would also reduce capillary blood flow by virtue of the great fall in systemic blood pressure.

D. Neurotransmitters of Vasomotion

In an interesting study of the chemical transmitters released in the submaxillary gland after stimulation of the chorda tympani and the sympathetic nerves, Cattell, Wolff and Clark (1934) demonstrated that the sympathetic and parasympathetic transmitters were different. Using the denervated nictitating membrane as a bioassay system, they concluded that sympathetic stimulation liberates an adrenaline-like substance. Cocaine was found to enhance the

contraction of the denervated nictitating membrane *in vitro*; *in vivo* it increased the magnitude of the sympathetic vasoconstriction in both normal and sympathetically denervated glands. Cattell made an additional observation, viz; that the secretory response to sympathetic stimulation diminished progressively in the course of successive periods of stimulation. As the secretory response faded away, the sympathetic vasodilatation also disappeared. The sympathetic vasoconstriction, however, was observed as before, indicating that it was independent of secretion.

Oborin (1952) investigated the effects of the stimulation of cervical sympathetic nerves on blood flow in the submaxillary glands of the cat. He found that weak sympathetic stimulation evoked a transient, small dilatation; this response could also be duplicated by a threshold dose of adrenaline or by sympathetic nerve stimulation after a small dose of dihydroergotamine. The response to stronger stimulation was characterized by a prominent vasodilatation that was always preceded by a period of vasoconstriction; a large dose of adrenaline produced a similar response.

Another interesting finding of Oborin's was the detection of a sympathomimetic substance in the venous blood leaving the gland after sympathetic stimulation. Evidence that the substance was not noradrenaline but could have been adrenaline was shown by parallel assays on the nonpregnant rat uterus and the rat colon. Oborin concluded that the sympathetic vasodilatation was mainly a reactive hyperemia, since short-term occlusion of the arterial supply was followed by a dilatation similar to that seen on sympathetic

stimulation. A minor contribution to this reactive hyperemia was thought to be due to the presence of a weak, adrenergic vasodilator component.

E. α and β Adrenergic Receptors (Adrenoceptors)

In 1948, Ahlquist reported his classical study which consolidated the evidence for different classes of adrenoceptors. Dale (1906) had provided the first evidence for different types of adrenoceptors when he used ergotoxine (a preparation of ergot alkaloids) to study the effects of sympathetic nerve stimulation and adrenaline. By 1910 Barger and Dale had shown that it was possible to differentiate adrenoceptors by two methods - selective blockade by antagonists and/or comparison of the relative potencies of a series of agonists, in this case sympathomimetic amines.

Ahlquist had used the latter method to differentiate among two classes of adrenoceptors in each of a variety of sympathetically innervated effector systems. Ahlquist chose the following sympathomimetic amines: adrenaline, noradrenaline, α-methyl-adrenaline, α-methyl-noradrenaline, and isoprenaline. One of the systems he studied was the smooth muscle of peripheral blood vessels, and it is of interest to examine his findings. For vasoconstriction, the relative potencies in increasing order were: isoprenaline, α-methyl-adrenaline, α-methyl-noradrenaline, noradrenaline, adrenaline. For vasodilatation the order (again in increasing relative potency) was markedly different: noradrenaline, adrenaline, α-methyl-adrenaline, α-methyl-noradrenaline, isoprenaline. Thus adrenaline proved to be the most potent vasoconstrictor and isoprenaline the most potent vasodilator.

From this data and the data obtained from other systems, Ahlquist concluded that these two distinct orders of potencies reflected the properties of two different classes of adrenoceptors. Ahlquist named these two adrenoceptor types alpha and beta. He concluded that in the vascular smooth muscle system the alpha adrenoceptor mediated vasoconstriction, whereas the beta adrenoceptor mediated vasodilatation.

The effects of adrenaline and noradrenaline on blood flow in normal and chronically denervated cat submaxillary glands were examined by Graham and Stavraky (1954). These investigators established that a peculiar dose-response relationship existed with respect to the effects of adrenaline and noradrenaline on normal and sympathetically denervated glands. Small doses of both adrenaline and noradrenaline produced vasodilatation in normal submaxillary glands as well as in glands denervated by extirpation of the superior cervical ganglion. The only difference between normal and denervated glands was that the denervated gland, being sensitized, had a much lower threshold. For large doses of these agents the response was vasoconstriction; again, normal and denervated glands differed only with respect to the threshold dose.

In a series of investigations, Emmelin (1955a, b) explored the interrelationship between blood flow and secretion as well as between the parasympathetic and sympathetic systems in the submaxillary gland of the cat. He examined the effects of various sympathicolytic agents on the secretory and vascular effects of sympathetic stimulation; his findings may be summarized as follows:

1. Sympathetic nerve stimulation resulted in constriction followed by dilatation when stimulation was discontinued.
2. Stimulation of the sympathetic nerves during a period of chorda-induced hyperemia in most cases reduced the magnitude of vasodilatation. However, sympathetic nerve stimulation sometimes failed to reduce the chorda dilatation - even when the constriction produced by sympathetic nerve stimulation alone was very pronounced.
3. In some cats in which the sympathetic after-dilatation was abolished with chlorpromazine, the sympathetic vasoconstriction overcame the chorda-induced hyperemia.
4. When the sympathetic response was seen, that of a vasoconstriction preceding vasodilatation, then the concomitant reduction in the rate of secretion of saliva could be attributed to the vasoconstriction.
5. In rare instances after administration of Regitin (Phentolamine), an α-adrenoceptor antagonist, sympathetic nerve stimulation produced two or more successive periods of vasodilatation. The first dilatation occurred shortly after the beginning of stimulation and the last after cessation of stimulation.
6. Chlorpromazine left only the vasoconstrictor responses to sympathetic nerve stimulation; a ganglionic action was eliminated since both pre- and postganglionic stimulation produced the same response. Chlorpromazine also abolished the secretory responses to injected adrenaline

and noradrenaline.

7. Priscol (Tolazoline), a known blocker of α-adrenoceptors, blocked the sympathetic vasoconstriction, thereby making the vasodilatation appear pronounced. Secretion was generally unaffected.

From these complex observations Emmelin concluded that the sympathetic vasodilatation is closely connected with secretion and is probably due to metabolites having vasodilator actions. This conclusion was a reaffirmation of the views expressed earlier by Barcroft (1914).

F. The Role of Kallikrein in Mediating Vasodilatation in the Submaxillary Gland

In 1955, Hilton and Lewis, taking up the suggestion of Ungar and Parrot (1936) began publishing a series of reports (Hilton and Lewis, 1955a, b; 1956; 1957) supporting the theory that kallikrein was indeed the long sought-after mediator of the atropine-resistant chorda dilatation. Thus, in their view it was superfluous to invoke the existence of parasympathetic dilator fibres to the submaxillary glands. Hilton and Lewis developed the generalization that functional vasodilatation in most organs is mediated by kallikrein.

The hypothesis put forward by Hilton and Lewis alleges that during secretory activity, an enzyme, kallikrein, is secreted from the secretory cells of the submaxillary gland into the interstitial fluid. There it cleaves a plasma protein, an α-2-globulin, to release the vasoactive decapaptide, kallidin. The following observations were cited as proof for this hypothesis:

1. In a perfused cat submaxillary gland preparation, kallikrein appears in the venous perfusate after chorda tympani nerve stimulation.
2. Close-arterial injection of atropine prevented the appearance of kallikrein in the perfusate; atropine also does not block nerve-induced vasodilatation.
3. If, during the first fifteen seconds of a one-minute arterial conclusion, the chorda tympani nerve is stimulated, then a protracted dilatation is seen with restoration of the normal arterial flow.

Hilton and Lewis therefore concluded that kallikrein, by causing formation of bradykinin, was the specific chemical mediator of chorda vasodilatation. Almost as an afterthought, Hilton and Lewis broadened their kallikrein-kinin hypothesis of functional vasodilatation so as to include the after-dilatation seen on cessation of sympathetic stimulation.

Bhoola, Morley and Schachter were the first to dispute the Hilton-Lewis formulations when, at the Cambridge meeting of the Physiological Society in September of 1962, they presented the following findings:

1. The time course and magnitude of the vasodilatation resulting from the close-arterial injection of dialyzed cat saliva differs significantly from that elicited by chorda stimulation or close-arterial injection of acetylcholine. The latter two are quicker in onset, are greater in magnitude and less prolonged.

2. Intra-arterial administration of large doses of bradykinin or dialyzed cat saliva resulted in the desensitization of the glandular vessels to these agents. However, during this period of desensitization to bradykinin, the equivalent vasodilator responses to chorda stimulation or close-arterial injections of acetylcholine were little affected.
3. When the gland was perfused with horse serum, from which cat saliva does not release bradykinin, chorda stimulation still resulted in a maximal vasodilatation.

These results were not in accord with the view that the kallikrein-kinin system does mediate the chorda hyperemia, and Bhoola *et al.* maintained that the concept of parasympathetic vasodilator fibres was still acceptable.

In 1965, Bhoola, Schachter and Smaje examined the effects of α- and β-adrenoceptor antagonists and guanethidine on the sympathetic after-dilatation and observed the following:

1. α-adrenoceptor antagonists converted the sympathetic after-dilatation to an immediate one, that is the dilatation now occurred during sympathetic stimulation instead of upon its cessation. Tolazoline was the most effective antagonist.
2. β-adrenoceptor antagonists reduced or abolished the sympathetic after-dilatation, leaving the vasoconstriction unaffected or slightly enhanced.
3. A concomitant effect of β-blockers was a reduction in

secretion; α-blockers had a much more varied effect. AT-3 blocked the secretion, phenoxybenzamine in high doses blocked it, and tolazoline either increased it or left it unaffected.

4. High doses of α- or β-antagonists abolished all responses seen on sympathetic stimulation.
5. Guanethidine blocked all sympathetically mediated responses.

This set of observations led Bhoola *et al.* to the conclusion that the secretory, constrictor and vasodilator responses due to sympathetic nerve stimulation were all related to the release of noradrenaline and/or adrenaline. Furthermore, since secretion and vasoconstriction were separable by the actions of α-adrenoceptor antagonists, such as tolazoline, they concluded that separate sympathetic secretory and vasoconstrictor fibres existed. The sympathetic after-dilatation, it was concluded, was most probably due to the action of noradrenaline on vascular β-adrenoceptors, not an unreasonable view in light of the results of differential adrenoceptor blockade.

The following year, Morley, Schachter and Smaje (1966) examined vasodilatation in the submaxillary gland of the rabbit; in this animal, however, the chorda vasodilatation was atropine sensitive. The sympathetic after-dilatation was significantly different from that in the cat. Unlike that in the cat, in the rabbit it was small and variable. Also, on no occasion after α-adrenoceptor blockade was there conversion of the after-dilatation to an immediate

one, even though differential adrenoceptor blockade again separated the component responses (secretion and vasodilatation) to sympathetic nerve stimulation. No conclusions could be drawn about the possible nature of the sympathetic neurotransmitter.

Beilenson, Schachter and Smaje (1968) further explored the relationship of kallikrein to its alleged role in vasodilatation in submaxillary glands. Their findings may be summarized as follows:

1. Kallikrein was secreted in highest concentrations in saliva obtained on sympathetic nerve stimulation; chorda saliva contained kallikrein in much lower concentrations. Prolonged stimulation of the chorda tympani resulted in a progressive decrease in the kallikrein content of the saliva.
2. Duct ligation reduced the kallikrein content of the cat submaxillary gland by approximately ninety-five percent. Glands that had been depleted of their kallikrein content by duct ligation plus sympathetic nerve stimulation nevertheless responded to chorda stimulation with a normal dilatation.
3. There was a close parallelism between the rate of secretion of saliva and the chorda dilatation over a wide range of frequencies of stimulation. However, there was no correlation between chorda dilatation and the kallikrein concentration of the saliva at different frequencies.

From these results, they concluded that parasympathetic vasodilator fibres to the submaxillary glands probably existed and that the kallikrein-kinin system played no significant role in chorda-induced vasodilatation.

Further evidence for the existence of parasympathetic vasodilator fibres to the submaxillary gland of the cat was presented by Karpinski, Barton (nee Beilenson) and Schachter (1971). Using a new type of flow meter which minimized mechanical delay in recording flow changes, it was found that the onset of vasodilatation was separated from the beginning of nerve stimulation by a latency as short as 350 milliseconds. Creed and Wilson (1969) had arrived at a mean latency value between nerve stimulation and secretory cell hyperpolarization of 300 milliseconds. Therefore, the results of Karpinski *et al.* indicated that the kallikrein-kinin system could not be involved in the initiation of chorda vasodilatation because of the short latency. A more plausible explanation was the existence of vasodilator fibres in close proximity to blood vessels and acting directly on them. This conclusion was not inconsistent with the electron microscopic findings of Garrett (1966a, b), who observed nerve endings in close proximity to glandular vessels. The possibility that some of these nerve endings were not parasympathetic but were of sympathetic origin was not excluded.

In 1972, Gautvik, Kriz and Lund-Larsen reinvestigated the role of plasma kinins in relation to the sympathetic after-dilatation. Their experiments were designed to examine in a more conclusive way whether or not this was mediated by plasma kinins. By alternating

periods of perfusion with normal and kininogen-free perfusate (adsorbed horse plasma) the following observations were made:

1. During perfusion with kininogen-free substrate, the vasodilatation gradually disappeared.
2. The vasodilatation, however, could be restored by close-arterial injection of noradrenaline after addition of kininogen to the perfusion medium.

From these observations Gautvik *et al.* concluded that kinins, by acting on vascular smooth muscle, oppose the initial vasoconstriction and then cause the after-dilatation. They did not, however, completely exclude the possible contribution of vascular β-adrenoceptors to the sympathetic after-dilatation. This work may be criticized on the grounds that it bears little relevance to the study of the sympathetic after-dilatation as sympathetic nerve stimulation was not performed throughout the experiments.

In a later communication, Gautvik *et al.* (1974) re-examined the possible contribution of β-adrenoceptors, as well as kinins, to the sympathetic vasodilatation. Using agents that either block or stimulate adrenoceptors, they examined kallikrein secretion in relation to nerve-induced vasodilatation and found the following:

1. Whereas sympathetic nerve stimulation and close-arterial injections of noradrenaline decreased glandular kallikrein (as measured by hydrolysis of α-N-Benzoyl-L-

Arginine-Ethyl ester in glandular homogenates), isoprenaline, a β-adrenoceptor agonist, failed to produce kallikrein secretion.

2. Secretion of kallikrein in response to sympathetic nerve stimulation was blocked with the α-adrenoceptor antagonist, Regitin; in addition, vasoconstriction was abolished, and the sympathetic after-dilatation markedly reduced.
3. Propanolol, a β-adrenoceptor antagonist, reduced the magnitude of vasodilatation seen after sympathetic nerve stimulation, but did not completely block it.

These findings led Gautvik *et al.* to modify their previous view and conclude that the sympathetic after-dilatation is due to a combination of two mechanisms. In their opinion, the vasodilatation seen after sympathetic nerve stimulation could be attributed to direct stimulation of vascular β-adrenoceptors, and α-adrenoceptor-mediated secretion of kallikrein, leading to intraglandular kinin formation.

Schachter, Barton and Karpinski (1973a, b) adopted a different experimental approach to that of Gautvik *et al.* If kinins did indeed play a role in either chorda or sympathetic vasodilatation, then their effects should be potentiated by the synthetic nonapeptide, Bradykinin potentiating factor (BPF). This was found NOT to be the case, since BPF did not affect either the chorda hyperemia or the sympathetic after-dilatation. With this evidence, it becomes increasingly difficult to accept the view that

the kallikrein-kinin system is a major mediator of functional hyperemia in the submaxillary gland.

In a further report, Schachter, Barton and Karpinski (1973b) found that the sympathetic after-dilatation was enhanced after chronic parasympathetic denervation. Also, under these conditions vasodilatation occasionally appeared immediately on sympathetic stimulation. The sympathetic vasoconstriction did not appear to be affected as a result of degenerative section of the chorda tympani. BPF, as with the normal after-dilatation, had no effect on the enhanced sympathetic after-dilatation. Schachter *et al.* concluded that the mechanism underlying the normal sympathetic after-dilatation, and its subsequent enhancement after chronic parasympathetic denervation remained unexplained. Since the normal and enhanced after-dilatation appeared to be unaffected by doses of atropine and propanolol, which blocked muscarinic cholinoceptors and β-adrenoceptors respectively, it seems unlikely that the enhanced after-dilatation is due to proliferation of β-adrenoceptors.

G. Other Aspects

Davey and co-workers (1965) investigated blood flow through the submaxillary glands of cats pretreated with drugs that interfered with the normal synthesis, storage, or release of catecholamines in sympathetic nerves. Using bretylium, guanoxan, guanethidine and reserpine, they made the following observations:

1. The vasoconstrictor and secretory responses to sympathetic nerve stimulation were abolished by bretylium. This drug also reduced the vasodilator

and secretory responses to chorda stimulation. Although the mechanism of action of bretylium remains obscure even at the time of this writing, it is known that this agent prevents release of noradrenaline. Bretylium has no depleting action on catecholamines stored in nerve terminals.

2. Close-arterial administration of guanethidine in small doses abolished both the secretory and vasodilator responses to sympathetic stimulation. The sympathetic vasoconstriction, however, required larger doses to be blocked. Guanethidine, unlike bretylium, decreases sympathetic activity by depleting noradrenaline stores. Additionally, like bretylium, it also rapidly blocks sympathetic activity by preventing transmitter release.
3. Cats that had been pretreated with reserpine twenty-four hours earlier showed the usual vasodilatation on chorda stimulation; all responses to sympathetic stimulation were abolished. Reserpine depletes noradrenaline, dopamine and serotonin from various binding sites in a variety of ways; it interferes with granular uptake mechanisms and it may interfere directly with amine synthesis (Shore, 1972).
4. The sympathetic after-dilatation was blocked by the β-adrenoceptor antagonist, pronethalol.

From these observations Davey *et al.* concluded that although the chorda vasodilatation was abolished by adrenergic blocking agents,

these effects were nonspecific and probably related to the antagonistic effects on cholinergic ganglionic transmission. The chorda vasodilatation was not adrenergic, as reserpinization did not affect it. An adrenergic mechanism, however, not a plasma kinin, appeared to be responsible, in their opinion, for the sympathetic after-dilatation.

In 1968, Skinner and Webster also concluded that kinins were not involved in mediating the chorda hyperemia in the cat. The hyperemia produced by close-arterial infusion of bradykinin could be blocked if the perfusing blood contained carboxypeptidase B, an inactivator of bradykinin; however, the hyperemia seen on chorda tympani stimulation was unaffected. Skinner and Webster also observed that the β-adrenoceptor antagonists, propanolol and dichloroisoprenaline, reduced the chorda dilatation as well as blocking the hyperemia seen on injection of the β-adrenoceptor agonist, isoprenaline. Therefore, they suggested that the chorda dilatation could be accounted for on the basis of activation of both cholinoceptors and β-adrenoceptors in the blood vessels, i.e., that chorda fibres were at least, in part, adrenergic.

Schachter and Beilenson (1968) questioned this view. Although they confirmed the existence of vascular β-adrenoceptors in the submaxillary gland, they observed that propanolol also reduced the vasodilator responses to close-arterial injections of acetylcholine. An adrenergic component in the chorda dilatation was ruled out since they showed that the reserpinization did not affect the chorda hyperemia but eliminated all secretory and vascular effects

of sympathetic nerve stimulation in the submaxillary gland. Such had also been the case in the work of Davey *et al.* (1965). Schachter and Beilenson concluded that propranolol had an anti-cholinergic action, which explained the reduction in vasodilatation after chorda stimulation.

An entirely different approach to the study of parasympathetic vasodilatation was taken by Lundvall and Holmberg (1974), who examined the relationship of tissue hyperosmolality and parasympathetic vasodilatation in the cat submaxillary gland. Noting that hyperosmolality is an important mediator of exercise hyperemia in active skeletal muscle (Lundvall, 1972), these workers showed that a similar mechanism contributes, in part, to the vasodilatation seen in response to chorda nerve stimulation. A further investigation into the role of hyperosmolality in the sympathetic afterdilatation (Holmberg and Lundvall, 1976) showed that tissue hyperosmolality is an important causal factor in nerve-induced dilatation, especially at low and moderate stimulation frequencies (2-16 Hz). As β-adrenoceptor blockade with propanolol reduced both vasodilator and hyperosmolar responses associated with sympathetic nerve stimulation, Holmberg and Lundvall suggested that β-adrenoceptor vasodilatation could be linked to metabolism. The role of sympathetic vasodilator fibre mechanisms was seen as being contributory to the net response to nerve stimulation.

H. Summary and Objectives of Present Work

It appears, then, that the question of parasympathetic vasodilator nerve fibres has gone full circle to the original views

of Claude Bernard. The possibility clearly exists that there are specific parasympathetic vasodilator nerve fibres, despite their relative resistance to atropine. This view would make the idea of different cholinoceptors in the secretory and vascular elements of the salivary gland analogous to that of the different adrenoceptors in various tissues.

Since the mechanism of the sympathetic after-dilatation remains obscure, the present study was designed to investigate it more closely. The objective of this work was to examine the effects of sympathetic nerve stimulation on blood flow through the submaxillary gland of the cat and rabbit with particular emphasis on the after-dilatation. Specifically, the following possibilities were examined:

1. The vasoconstriction and the after-dilatation may be due to different fibres in the sympathetic nerves.
2. The sympathetic after-dilatation may be due to stimulation of vascular β-adrenoceptors by noradrenaline released from the same postganglionic sympathetic fibres that are responsible for constriction.
3. The kallikrein-kinin system could in some way mediate the after-dilatation.
4. The after-dilatation may be an effect resulting from changes in the microcirculation, either as a mechanical response of arteriolar smooth muscle to the preceding vasoconstriction, or as a result of changes in the pattern of blood flow through the gland.

II. MATERIALS AND METHODS

A. Animals

Cats of both sexes weighing 1.9 to 5.3 kg. were used. Experiments were all acute with the exception of one cat pretreated with indomethacin and two in which the chorda lingual nerve was chronically sectioned. Rabbits of both sexes, weighing 2.4 to 5.1 kg. were also used.

B. Anaesthesia

Cats were initially anaesthetized by an oxygen-chloroform mixture that was piped into a closed box containing the animal. Once a cat was secured on the operating table, an ether-soaked mask was used to maintain anaesthesia until a cannula could be inserted into a femoral vein. When the venous cannula was in place, alpha-chloralose (40-80 mg kg^{-1} I.V.) was administered to effect a suitable depth of anaesthesia throughout the experiment.

Rabbits were anaesthetized by intravenous injection of urethane (2 gm kg^{-1}) through a marginal ear vein. More urethane was administered as necessary via a cannula in a femoral vein.

C. Preparative Surgery

Tracheotomy was routinely performed in all acute experiments and a glass or metal cannula inserted to allow for artificial ventilation if necessary. Blood pressure was recorded via an arterial cannula in either the carotid or femoral artery. This cannula was then connected to a precalibrated arterial pressure transducer.

D. Nerve Stimulation

The chorda tympani was exposed cephalad to the hilus of the submaxillary gland in the region of the sublingual and submaxillary ducts. It was then cut central to the branch point with the chorda lingual nerve. Parasympathetic stimulation was mainly used as a check on the functional integrity of the vascular bed as seen by the dilator response.

For sympathetic stimulation it was first necessary to expose the sympathetic nerve trunk as it ran in the vagosympathetic trunk in the neck. The component fibre tracts of this trunk were separated from each other and the preganglionic sympathetic supply was identified by the effect of electrical stimulation on pupillary dilatation (mydriasis). In the two instances that postganglionic stimulation was employed the preganglionic trunk was first traced to the superior cervical ganglion. By the electrical stimulation of various ganglionic branches, it was possible to identify the branch innervating the gland by the change in blood flow.

Once nerves had been dissected and cut, their distal ends were mounted on bipolar platinum electrodes. Paraffin oil was used to cover the nerve-electrode assemblies and thus prevent dehydration while ensuring proper insulation from the surrounding tissue.

Chorda stimulations were usually supramaximal; 7 volts at 15 Hz with a pulse duration of 0.4 msec; train length was variable. Stimulation of the cervical sympathetic nerves was more complex; the different stimulus parameters and their ranges are listed in Table 1. Electrical stimulation was carried out using a two-channel

Table 1. Different Stimulus Parameters Used in Sympathetic Nerve Stimulation

Type of Stimulation	
Voltages	2.25 - 50 V
Frequencies	1 - 50 Hz
Pulse Durations	0.05 msec - 0.5 msec
Train Lengths	1 sec - 60 sec

Digitimer (W.P. Instruments) coupled with two stimulus isolation units; an induction coil was used for stimulation in one of the experiments.

E. Blood Flow

Blood flow was measured by monitoring the venous outflow from the submaxillary gland using a forced convection flowmeter. The flowmeter probe was inserted into an external jugular vein by a double cannulation after all the veins draining into the external jugular (with the exception of the submaxillary vein) had been tied off. Sodium heparin (5 - 10 mg/kg I.V.) was administered to prevent formation of clots at the thermistors within the probe. The probe had been previously siliconized to present a non-wetting surface. A pictorial representation of the experimental set-up used in blood flow experiments is given by Fig. 1.

F. Measurement of Duct Pressure

The submaxillary gland duct (which lies under the mylohyloid muscle and medial to the duct of the sublingual gland) was exposed and cannulated with fine polyethylene tubing at a point cephalad to its emergence in the floor of the mouth. A three-way stopcock was fitted onto the flared plastic end of the cannula. To this stopcock a narrow bore tube was also connected. A pre-calibrated venous pressure transducer was connected to the remaining stopcock end.

Positive pressures were created by filling the tube with saline to various heights; the greater the height of the saline column, the greater the pressure exerted. A negative pressure was

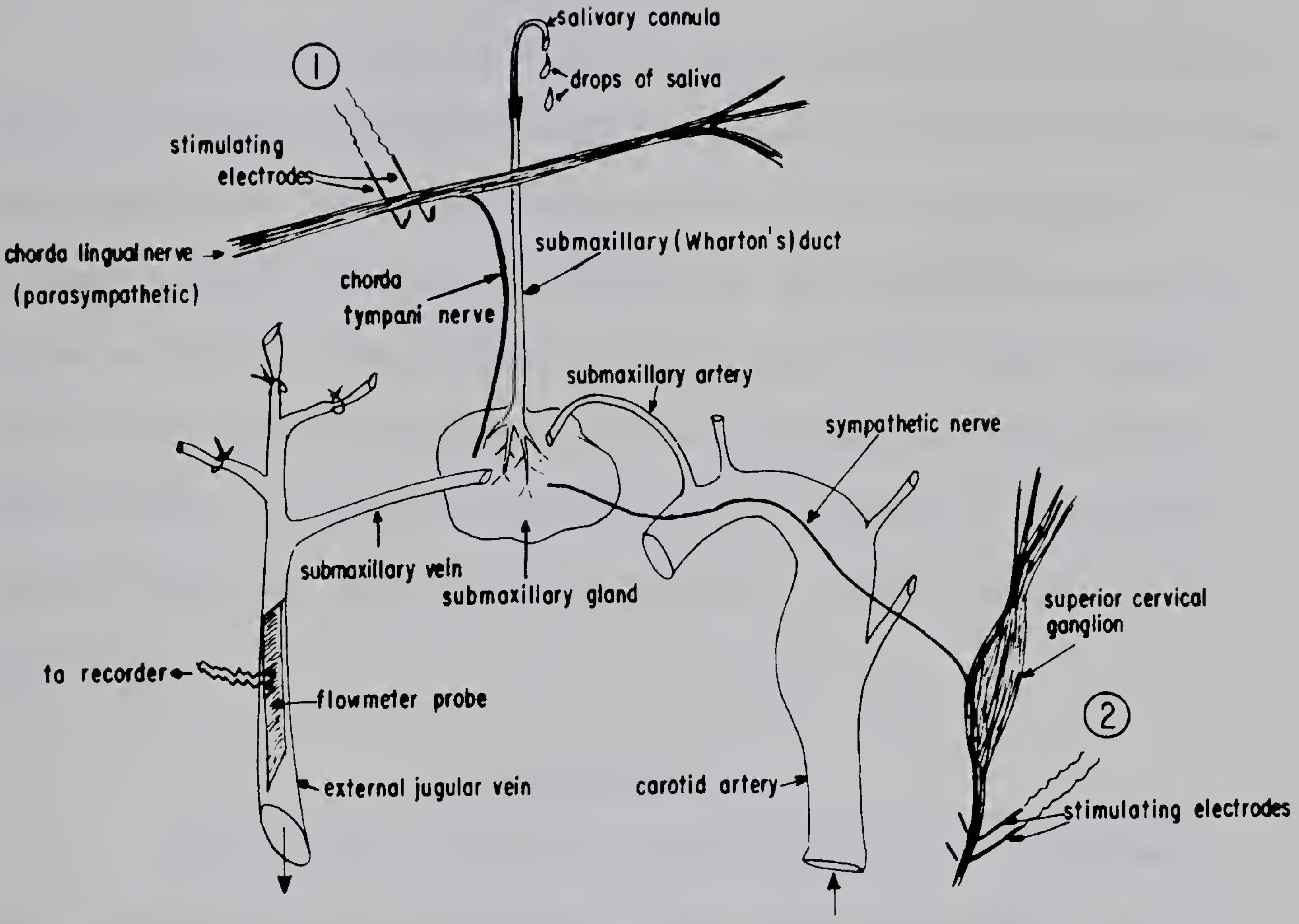

Fig. 1. Schematic representation of the experimental set-up for studying blood flow. Salivary secretion was not monitored; the duct is shown, however, as gland duct pressure was measured in some experiments.

created by lowering the tube to a level below that of the animal.

G. Recording

All recording was done on a four-channel Sanborn recorder (Hewlett-Packard) with heat-sensitive paper. An interval time marker was always used; one channel always served for the time record. A second channel was used for blood flow; the flowmeter output was connected to the corresponding amplifier unit. The third channel was used for arterial pressure; in two experiments, duct pressure was measured. A record of stimulation was recorded on the fourth channel which was wired to the synchronous external outputs of the Digitimer.

H. Denervation

Chronic parasympathetic decentralization was performed under pentobarbitone anaesthesia, using sterile technique. An incision was made approximately 1 cm lateral to the midline and over the mylohyloid muscle at a point halfway between the transverse vein and the tip of the jaw. The chorda tympani was cut approximately 1 cm from its junction with the chorda lingual nerve in the region of the submaxillary and sublingual ducts. Approximately 2 cm of the chorda lingual nerve along with the adjoining stump of the chorda tympani was then excised. The incision was then sutured. In the subsequent acute experiment, 12 days later, blood flow was measured in both the intact and denervated submaxillary glands. In total two cats were used in this experiment.

I. Drugs

The drugs used are listed in Table II along with the relevant details. Administration was either intravenous or close-arterial via retrograde injection through a fine plastic cannula inserted into the lingual artery. Close-arterial injections were delivered in a volume of 0.5 ml over a 20 second period.

One cat was pretreated with indomethacin (50 mg kg^{-1} I.P.) injected twice daily over a period of three days. Indomethacin was made up into a stock solution of 100 mg ml^{-1} in the form of a sodium salt; sodium carbonate was used to dissolve the indomethacin, as this drug is relatively insoluble at neutral pH.

J. Electron Microscopy of Sympathetic Nerves

The preganglionic sympathetic trunk of the cat was traced to the superior cervical ganglion, care being taken to avoid severing of the artery supplying the ganglion. Once the ganglion and its branches had been freed from the surrounding tissue, the fibres running to the gland were identified by electrical stimulation as outlined in Sec. D. The ganglion and its branches were then fixed in situ by dropping a solution of cold ($0^{o}C$) potassium phosphate buffered (pH 7.2) formaldehyde-glutaraldehyde solution (Karnovsky, 1965) on the tissue. After 5 minutes of fixation the tissues were excised. Further preparation of tissues for electron microscopy was done according to standard techniques as discussed by Pease (1960).

Thin sections were examined using a Philips 300 Electron Microscope; all photographs were taken on 35 mm film.

Table II. Pharmacological Agents Used in Experiments

Agent	Form	Supplier
Adrenaline	Bitartrate salt	Nutritional Biochemicals Corp.
Bradykinin	Triacetate salt	Schwarz-Mann Bioresearch
Bradykinin Potentiating Factor	Nonapeptide linked to PCA	Drs. L. Green and J. Stewart
Dibozane	As free base	MacNeil Laboratories
Heparin	Sulfate salt	Nutritional Biochemicals Corp.
Indomethacin	Sodium salt	Sigma Chemical Company
Isoprenaline	Bitartrate salt	Sigma Chemical Company
Noradrenaline	Bitartrate salt	Nutritional Biochemicals Corp.
Phenoxybenzamine	Hydrochloride salt	Smith, Kline and French
Phentolamine	Ethane sulfonate salt	CIBA
Tolazoline	Hydrochloride salt	CIBA

III. RESULTS

A. Vascular Responses to Nerve Stimulation in the Submaxillary Gland of the Cat and Rabbit

The vascular responses to parasympathetic (chorda tympani n.) and sympathetic nerve stimulation in the submaxillary glands of the cat and rabbit are shown in Fig. 2 and Fig. 3 respectively. The characteristic effects of sympathetic nerve stimulation in the cat, as shown in greater detail in Fig. 4, consist of two distinct components, a vasoconstriction followed by a dilatation - the sympathetic after-dilatation. The latency of vasoconstriction ranges from 0.9 sec to 2.1 sec; this range of values was determined from over one hundred observations drawn from seven experiments. The constriction reaches its maximum during stimulation and then remains relatively constant until the cessation of stimulation, at which time blood flow increases. This after-dilatation may reach a maximum value as high as 65% of the dilatation seen on chorda stimulation. It is unlikely that the after-dilatation can be attributed to reactive hyperemia as arterial occlusion for the same period of time as the constriction results in a markedly smaller increase in blood flow.

Unlike the cat, the sympathetic response in the rabbit differs in that there is no after-dilatation. After cessation of sympathetic stimulation there is a small increase in blood flow; the duration and the magnitude of increase in blood flow suggests reactive hyperemia compensating for the constriction. Five rabbits

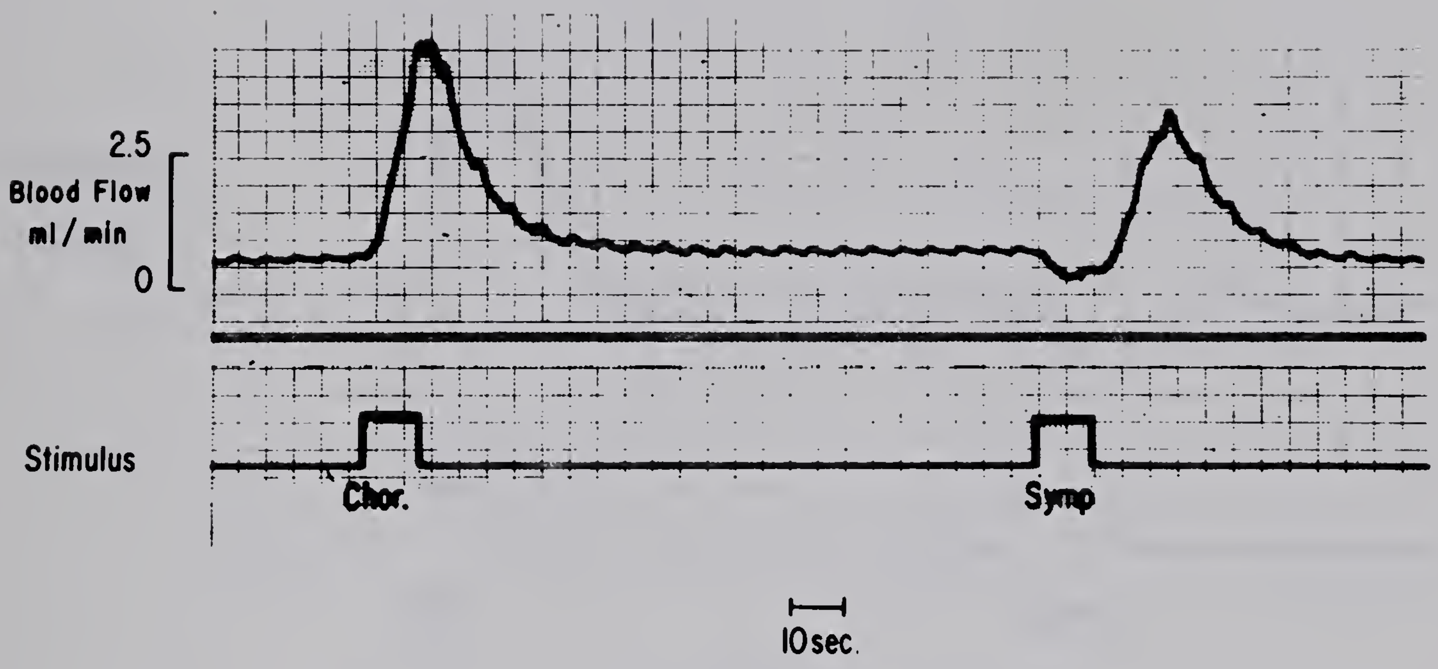

Fig. 2. Blood flow changes in the cat submaxillary gland seen in response to parasympathetic and sympathetic stimulation. Stimulation: 7.0 V, 25 Hz, 0.4 msec pulse duration for 10 sec.

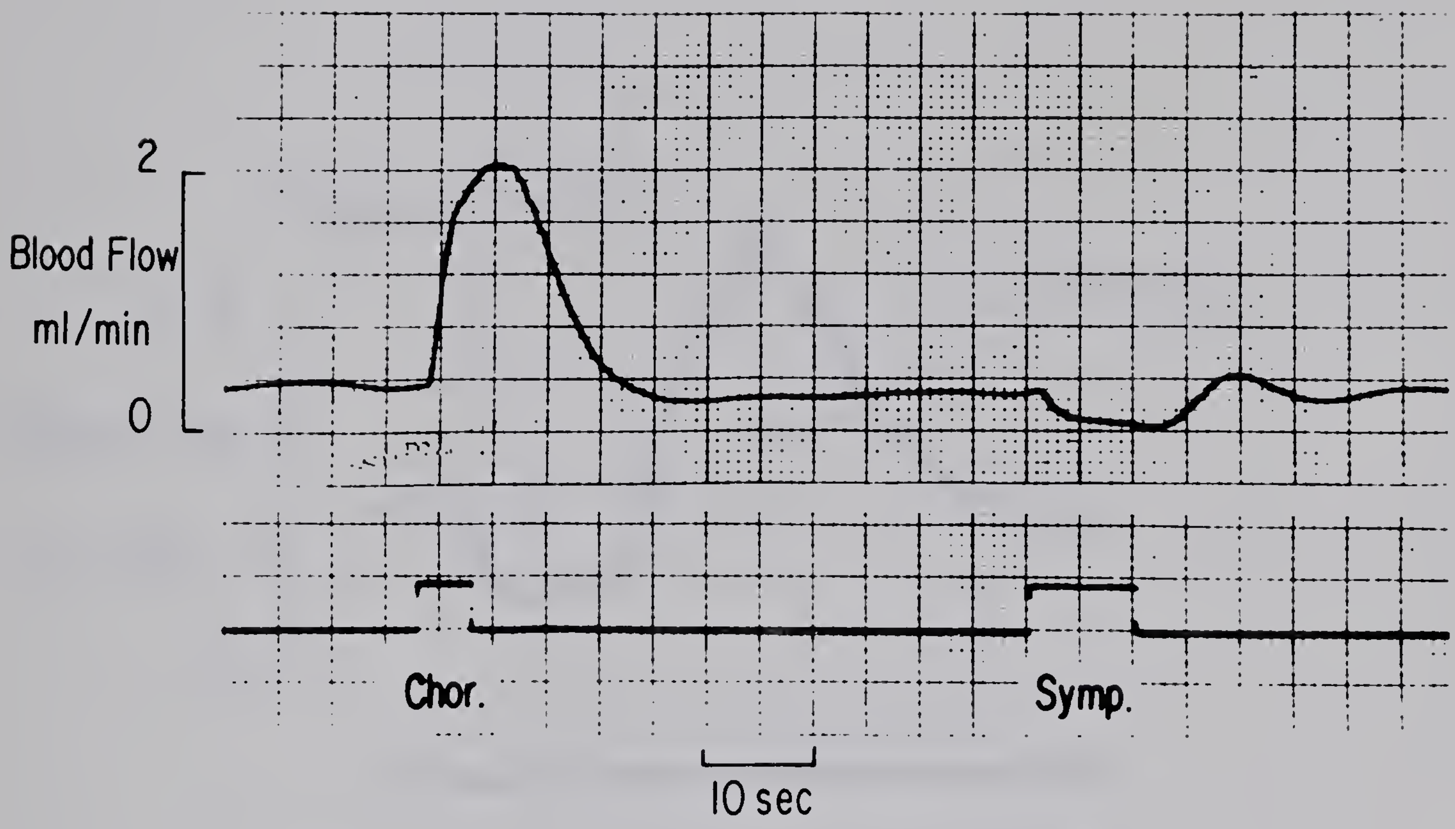

Fig. 3. Blood flow changes in the rabbit submaxillary gland seen in response to parasympathetic and sympathetic nerve stimulation. Stimulation parameters: 7.0 V at 25 Hz, 0.4 msec pulse duration for 5 (chorda) and 10 sec (symp.). The sympathetic response contains no after-dilatation as in the cat; the small, transient increase in blood flow seen upon cessation of stimulation may be attributed to reactive hyperemia.

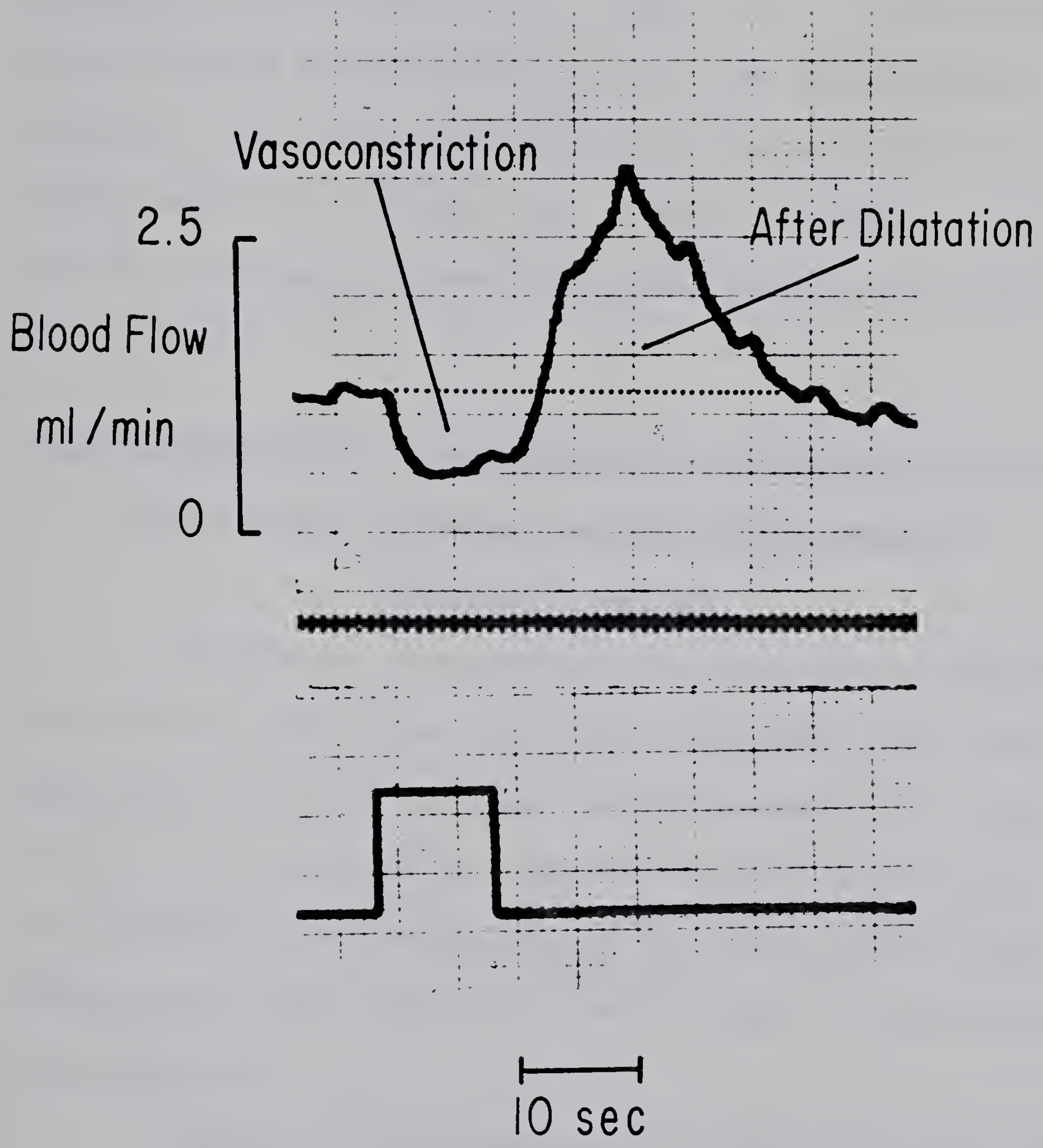

Fig. 4. Enlargement of the sympathetic response seen in Fig. 2. Details as in Fig. 2; the after-dilatation does not begin until cessation of nerve stimulation.

were examined and in no case was there any evidence for an after-dilatation. Varying the stimulus parameters also failed to produce an after-dilatation. It can be said with certainty then, that the rabbit does not display an after-dilatation in response to sympathetic nerve stimulation. Morley *et al.* (1966) had previously noted that the after-dilatation seen in the rabbit was small and variable. The greater accuracy of measurement obtained with the forced convection flowmeter used in these experiments would account for the discrepancy between the present findings and those of Morley *et al.*

B. The Relationship Between Stimulation Parameters Used For Sympathetic Nerve Stimulation and the Evoked Vascular Response in the Cat

Initially, experiments were performed to determine the threshold and supramaximal stimulus intensities; those results are presented in the next section. Having determined the stimulating voltage and pulse width that produced maximal vasodilatation and vasoconstriction, an examination of two other stimulus parameters, frequency and train length (i.e. total number of stimulating pulses), was carried out.

Changes in the magnitude of the after-dilatation were examined by using a fixed supramaximal stimulus (7.0 V) of constant pulse duration (0.4 msec) while randomly varying the frequency of sympathetic nerve stimulation at a constant train length. Increases in the frequency of nerve stimulation resulted in increases in the magnitude of the sympathetic after-dilatation in all experiments.

The relationship between the stimulating frequencies and the increases in blood flow appeared to be linear.

By varying the train length of sympathetic nerve stimulation in a random manner at a frequency of 20 Hz while keeping the other parameters constant (as defined in the preceding paragraph), it was possible to increase blood flow. Since basal flow rates differ between experimental animals and may affect the magnitudes of responses, observations pertaining to train length were confined to the two experiments cited above, in which both cats were the same age and weight. The effect of increasing stimulus train length (in other words, the number of stimuli) in both cases was the same, resulting in increases in blood flow. The increased flows corresponded in a linear fashion with the increasing stimulation times.

The parallel dependence of vasoconstriction and vasodilatation on the stimulation parameters used in sympathetic nerve stimulation suggests that, like the vasoconstriction, the after-dilatation is mediated by an adrenergic mechanism.

C. Experiments Concerning the Existence of Specific Dilator Nerve Fibres in the Cat Submaxillary Gland

1. Attempts to separate possible constrictor and dilator fibres by electrical stimulation of the sympathetic nerves of the cat.

Different types of electrical stimulation were used in attempts to separate constriction from dilatation. If the responses were mediated through two different groups of fibres of unequal diameter, separation should be possible. The smaller fibre, because

of its smaller cross-sectional area, would have a higher longitudinal resistance and would therefore require a larger stimulating voltage to reach its threshold than the larger, lower-resistance fibre. This difference could then be exploited to effect a separation of the responses due to two possible sets of nerve fibres. The response due to the larger fibres would then still be seen below the voltage threshold of the smaller fibres.

Attempts at separation were carried out in three experiments where stimulating voltages were varied by random assortment; the other stimulus parameters of frequency, pulse duration and train length were kept constant. No separation could be achieved. As the stimulating voltage increased, the magnitude of both constriction and dilatation also increased, as measured by the volume decrease or increase respectively. One set of results illustrating the voltage dependence of the two effects is shown in Fig. 5.

The volume increases of the sympathetic after-dilatation in response to increasing stimulus strengths (for the same range of stimulating voltages as used in Fig. 5) were measured as the areas under the dilatations, and therefore corresponded to the integrals of the flow rates. Increasing the stimulating voltage resulted in a proportionate increase in the volume of the after-dilatation, suggesting greater recruitment of vasomotor fibres as the stimulating voltage is increased. This observation is in agreement with results from other muscle systems. Attempts to confirm Carlson's (1907) observation that low-intensity short stimuli would selectively evoke vasodilatation were unsuccessful.

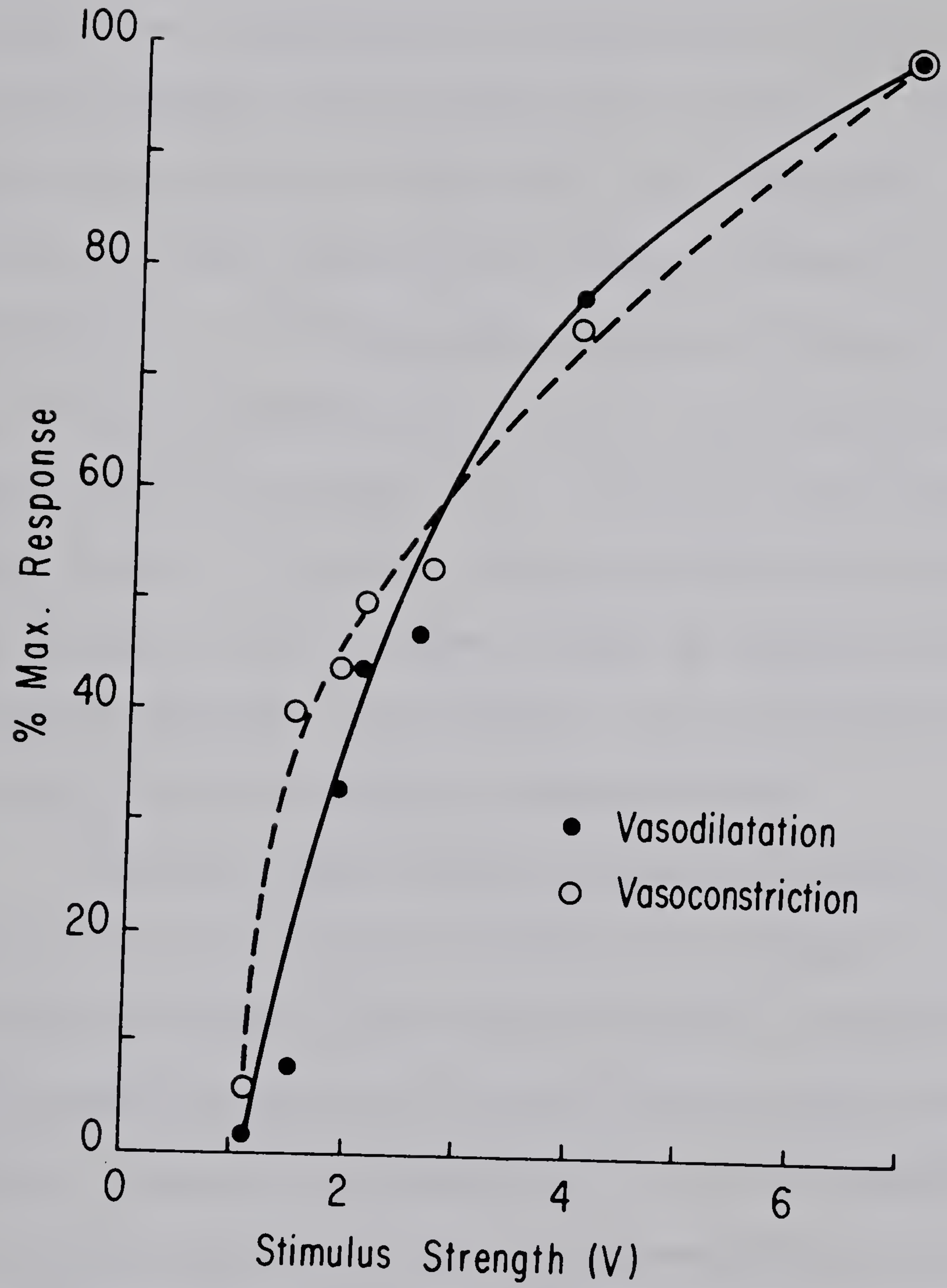

Fig. 5. Voltage-response relationships for paired vasoconstrictions and vasodilatations. Both curves follow each other very closely from threshold to maximal. Stimulation parameters: 0.4 msec pulse duration, 20 Hz for 10 sec.

To examine whether synaptic delay at the superior cervical ganglion could be a factor in the response to sympathetic nerve stimulation, a further attempt at separation was undertaken. Postganglionic sympathetic fibres were stimulated; no difference between pre- and postganglionic stimulation could be seen. Whether stimulation was preganglionic or postganglionic did not seem to affect the latency of the ensuing vasoconstriction. The latency between the cessation of sympathetic nerve stimulation and the start of the after-dilatation was also not affected by the site of stimulation. The relationship between the latency of vasoconstriction and the subsequent latency of the after-dilatation was relatively constant and did not appear to be affected by the location of the applied stimulus. Therefore, synaptic delay at the superior cervical ganglion would not appear to play an important role in the sympathetic response. The latency of the after-dilatation was generally twice the latency of vasoconstriction.

Carlson (1907) had reported distinct effects of pre- and postganglionic stimulation in animals given atropine. In the atropinized animal he found that preganglionic stimulation evoked slight vasodilatation only, and that postganglionic stimulation produced pronounced vasoconstriction. It is not possible to explain his findings on the basis of any known muscarinic action of atropine. However, the effects of atropine on this response were not investigated in these experiments.

2. Attempts to discover morphological differences among sympathetic unmyelinated fibres innervating the submaxillary gland of the cat.

Electron microscopy of pre- and postganglionic sympathetic nerves was carried out, with the aim of measuring unmyelinated fibre diameters; specimens from three cats were processed. Two hundred and eighty-five preganglionic and two hundred and ten postganglionic fibres were measured from photomicrographs; all measurements were taken to the nearest half of a millimeter. As the exact cutting orientation varied from fibre to fibre within the same sections, it was not always possible to measure the diameter of a perfect circle. Whenever an elliptical cross-section was encountered, the minor axis was taken as being equal to the true diameter of the axon. The measurements were converted to the actual size equivalent (in microns) by computation from the degree of magnification. Fig. 6, a photomicrograph, shows postganglionic fibres running from the superior cervical ganglion to the submaxillary gland of the cat.

The results are shown in Fig. 7 and it would appear that there are two different-sized fibres in the postganglionic population. Recent work by others (Williams et al., 1973) concurs in that there are distinct populations, of unmyelinated fibres differing in size, in the innervation of the superior cervical ganglion in the cat. If there are indeed two different-sized postganglionic fibres, the difference in fibre diameters would be so small so as to effectively preclude selective electrical stimulation of either fibre

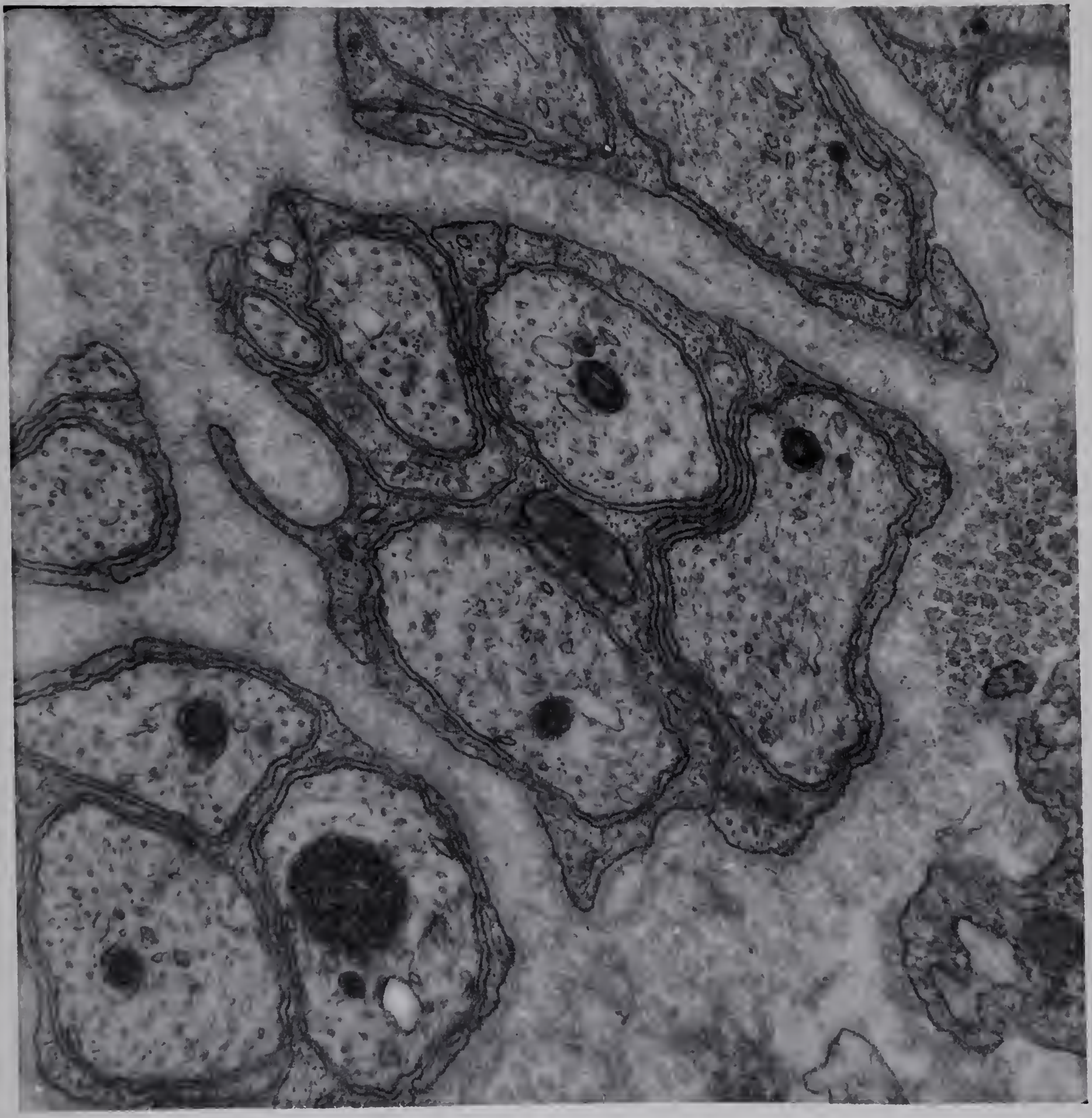

Fig. 6. Postganglionic fibres running to the submaxillary gland of the cat from the superior cervical ganglion (x37,100).

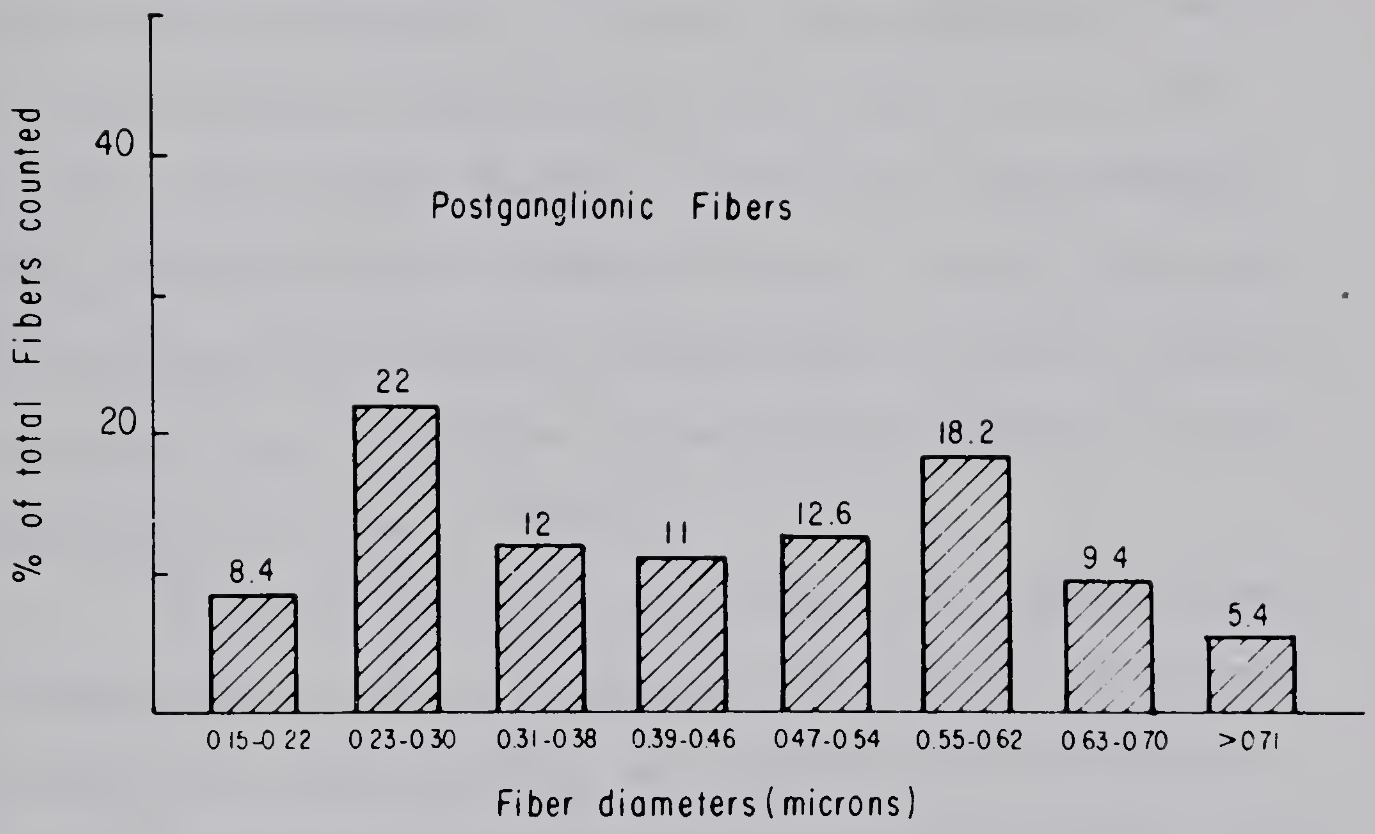

Fig. 7. Histogram of the size distribution of postganglionic unmyelinated fibres of the superior cervical ganglion of the cat.

3. Attempts to separate the vasoconstrictor and vasodilator responses by pharmacological means.

Administration of the α-adrenoceptor antagonist Dibozane in two experiments (2.5 mg kg^{-1} to a total dose of 10 mg) reduced the magnitude of both the sympathetic vasoconstriction and the after-dilatation. Examination showed the after-dilatation to be reduced following injection of Dibozane; this reduction, however, could be attributed to the systemic hypotension resulting from extensive α-adrenoceptor blockade. Comparison of the magnitude of the after-dilatation in relation to basal flow rate before and after α-adrenoceptor blockade indicated only a relative reduction. An additional dose of Dibozane reduced both responses to an even greater extent as shown in Fig. 8.

Tolazoline (dosage as per Bhoola *et al.*, 1965), another α-adrenoceptor antagonist, was administered in three experiments. It reduced the vasoconstriction but differed in its effect from Dibozane; the time course of the after-dilatation appeared altered. The after-dilatation seen after Tolazoline was characterized by a quicker onset as it appeared during a period of nerve stimulation rather than upon cessation of stimulation. On no occasion, however, did the after-dilatation appear immediately upon nerve stimulation. Tolazoline differs from the other α-adrenoceptor antagonists in that it enhances the secretory response of the submaxillary gland to sympathetic nerve stimulation; this enhancement of secretion had been previously noted by Emmelin (1955) and Bhoola *et al.* (1965).

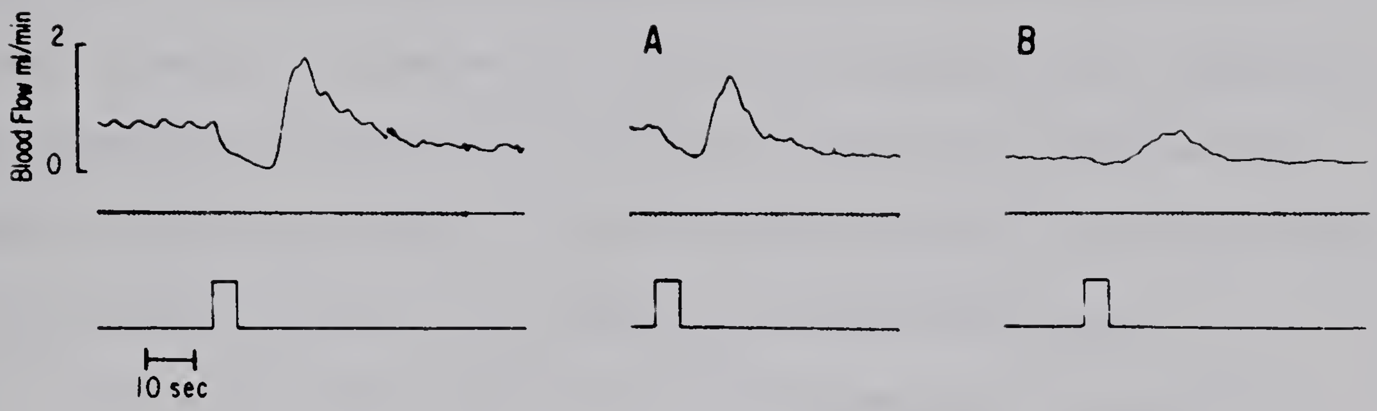

Figure 7. Effects of α-adrenoceptor blockade following administration of Dibozane. Stimulation parameters as per Fig. 2. At A Dibozane (1.5 mg kg^{-1} i.v. to a total of 5 mg) was injected. At B an additional 10 mg was given. The blood pressure, although not shown, dropped little following the first dose of Dibozane; after the second dose, however, it fell to approximately half the value of the resting blood pressure.

Indomethacin was employed to test the possibility that prostaglandins might mediate the after-dilatation; indomethacin inhibits prostaglandin synthetase, thereby lowering endogenous prostaglandin levels. Prostaglandins have also been implicated as inhibitors of noradrenaline release from postganglionic sympathetic nerve endings; in particular, attention has been directed toward prostaglandin E as a potential synaptic modulator (see Stjarne, 1973). A cat that had been pretreated with indomethacin displayed the same type of response to sympathetic nerve stimulation as did the other experimental animals. On the basis of this one experiment and considering the difficulty of obtaining a suitable control, the response seen must be viewed cautiously. It would seem, however, that indomethacin has no visible effect on the sympathetic after-dilatation.

To extend the comparative study between the cat and the rabbit, it was decided to examine more closely the question of what types of vascular receptors are present in the rabbit submaxillary gland. Several pharmacological agents were tested for activity; the main objective was to ascertain the existence of α- and β-adrenoceptors. Isoprenaline (0.5 - 1.0 μg close-arterially) was able to produce vasodilatation; it would appear, then that vascular β-adrenoceptors are present. Noradrenaline (0.2 - 2.0 μg close-arterially) and adrenaline (2.0 μg close-arterially) both produced vasoconstriction; pretreatment with the α-adrenoceptor antagonist Phentolamine (1 mg kg^{-1} I.V.) or Phenoxybenzamine (1.75 mg kg^{-1} I.V.) blocked this constrictor response. In addition, α-adrenoceptor blockade reduced the vasoconstriction seen in response to sympathetic nerve stimulation.

It is clear then that the vessels of the submaxillary gland of the rabbit have both α- and β-adrenoceptors and that the vasoconstriction in response to sympathetic nerve stimulation is probably mediated by vascular α-adrenoceptors. However, the degree of stimulation of vascular β-receptors remains obscure.

D. Does Kallikrein Play Any Role in the Sympathetic After-Dilatation?

The work of Schachter and his co-workers (1973a, b) excludes the possibility of kallikrein involvement in the case of the chorda dilatation in the cat as well as for the sympathetic after-dilatation. To add to previous observations, an experimental format identical to theirs was followed, and two experiments were performed on cats in which the chorda tympani had been sectioned 12 days earlier. The difference between the denervated and normal glands was as noted previously and is shown in Fig. 9. Besides the striking difference in the magnitudes of the after-dilatation, the latencies are significantly increased in the denervated gland. This was found generally to be the case in all records examined and would be expected on the basis of denervation supersensitivity.

Bradykinin potentiating factor (BPF, the nonapeptide) did not affect blood flow in response to sympathetic nerve stimulation in either the normal or denervated gland; this is in good agreement with the previous results obtained by Schachter *et al.* (1973b). BPF also did not affect the chorda dilatation in the control gland, again confirming previous findings that the kallikrein-

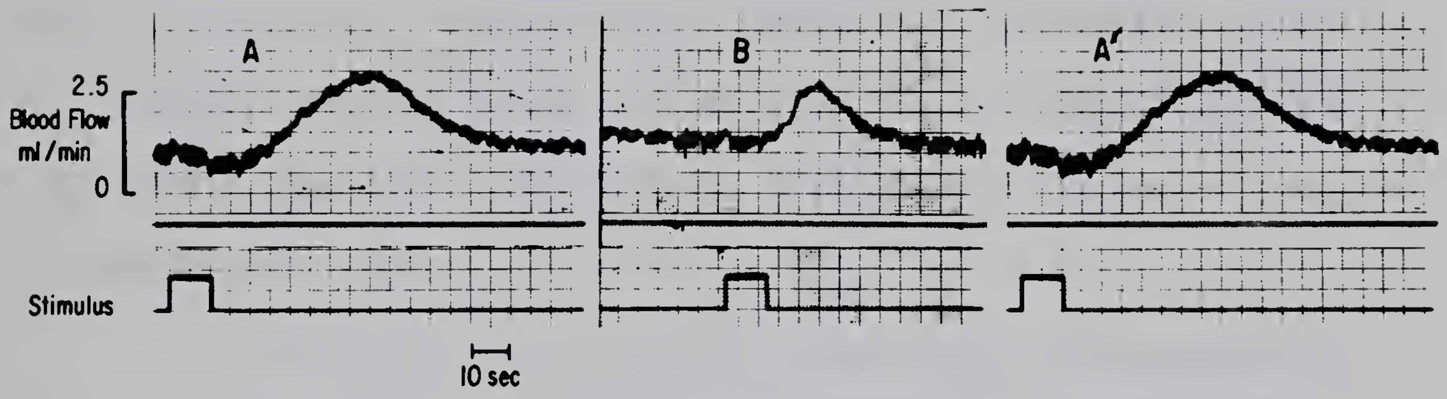

Fig. 9. Increased sympathetic response in the submaxillary gland of the cat following section of the chorda tympani. Record A was odtained from a denervated gland (chorda tympani sectioned twelve days earlier); B represents the response of the contralateral control gland. A' is another response recorded from the denervated side. Stimulation parameters as given in Fig. 2.

kinin system does not mediate the chorda response (Schachter *et al.*, 1973a).

E. Could the After-Dilatation Arise as a Hemodynamic Adjustment in the Microcirculation?

Emmelin *et al.* (1968) showed that a single stimulus applied to the chorda tympani produced vasodilatation in the submaxillary gland of the cat. This vasodilatation was accompanied by a pressure rise in the submaxillary duct, thus indicating myoepithelial cell contraction. Sympathetic stimulation caused no change in blood flow or duct pressure when a single stimulus was given; repetitive stimulation, however, did produce a pressure increase in the duct.

The possibility therefore arises that the sympathetic after-dilatation may be due to the contraction of myoepithelial cells. Myoepithelial cells have been seen surrounding the acini and intercalary ducts (Emmelin and Gjörstrup, 1974), as well as being adjacent to the blood vessels that run along the intralobular ducts within the gland; the presence of such multiple collateral circulation within the submaxillary gland has been known for some time (for a comprehensive synopsis of the submaxillary gland vasculature, the reader is referred to Burgen and Emmelin, 1961).

Under the above scheme the sequence of events might be as follows: Repetitive stimulation of the sympathetic nerves would result in vasoconstriction occurring at some site(s) in the arteriolar vasculature proximal to the collateral circulation in and around the ducts. Concomitant to this vasoconstriction,

myoepithelial cell excitation and contraction would take place; the vessels surrounded by the contracted myoepithelial cells would then dilate. This localized vasodilatation would not be detected as a change in the venous outflow because of the simultaneous dominant vasoconstriction. Upon cessation of sympathetic nerve stimulation, the vasoconstriction would quickly disappear while at the same time the myoepithelial cells would begin to relax. If the process of myoepithelial cell relaxation was slower than the smooth muscle cells in the blood vessels, collateral circulation would then still be in a state of dilatation relative to the resting state, i.e., before stimulation. The overall capacity of the glandular vasculature would be increased and would appear as the after-dilatation.

To explore the possibility raised above, negative and positive pressures were applied through the duct of the submaxillary gland while blood flow in response to sympathetic nerve stimulation was being recorded. Positive duct pressures might then hinder the relaxation of the myoepithelial cells, thereby prolonging the sympathetic after-dilatation. If negative pressures enhanced the relaxation process, then accordingly the after-dilatation would be reduced. Positive or negative pressures did not affect the responses seen on sympathetic nerve stimulation, as shown in Fig. 10.

The slight reductions seen in the after-dilatation in response to increasing positive duct pressures did not have a parallel in the corresponding vasoconstrictions, which were not affected. Furthermore, there were no changes in the basal flow

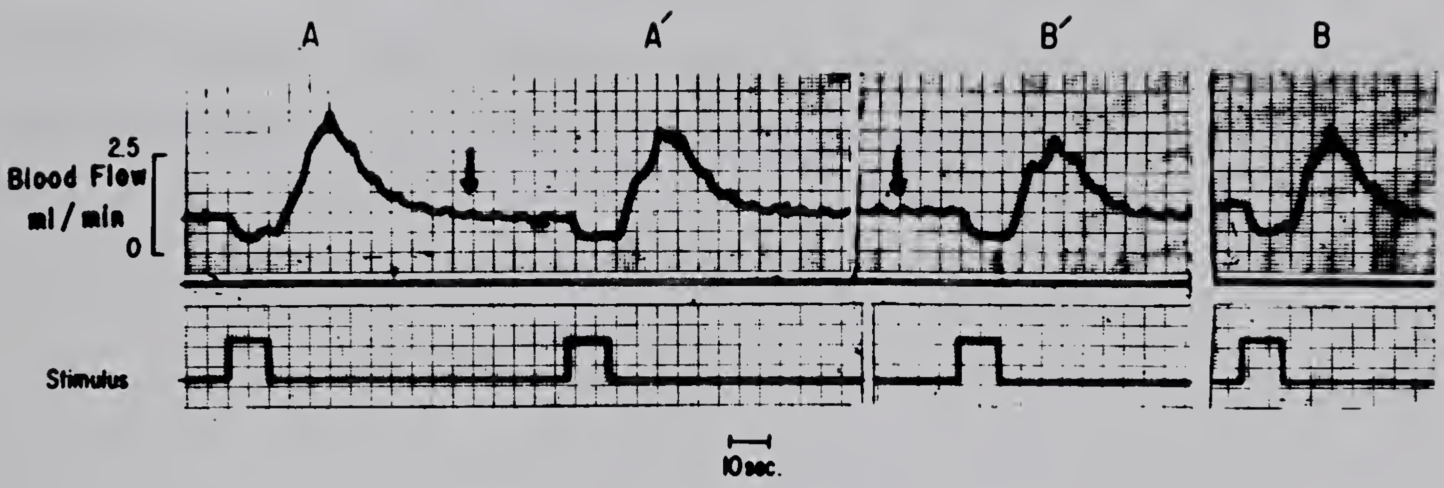

Fig. 10. Sympathetic responses during positive duct pressures. Preceding the response at A', a duct pressue equivalent to a column of 48 cm of water was applied; prior to B', this was increased to a column of 96 cm of water. The record at B shows the response after removal of pressure. There is little or no change in responses. Stimulation parameters: 7.0 V, 25 Hz, 0.4 msec pulse duration, for 10 sec.

rate. It would appear then that the after-dilatation cannot be attributed to mechanical, hemodynamic adjustments in the microcirculation of the gland. Although not conclusive, this would indicate that myoepithelial cells do not play a significant role in sympathetically mediated changes in the microcirculation of the glands. Unfortunately, the responses could not be assessed accurately over any length of time; after 20 minutes of interrupted positive duct pressures, glands became edematous very quickly, and thus unsuitable for further experimentation.

IV. DISCUSSION

More than sixty years has elapsed since Carlson reported the existence of sympathetic vasodilator nerve fibres in the cat submaxillary gland, and yet their presence cannot be conclusively demonstrated. Attempts at obtaining clear-cut separation of possible vasoconstrictor and vasodilator nerve fibre effects by differential electrical stimulation did not have any measure of success. However, on the basis of the experimental work cited in this thesis, it may be shown that the two effects display very close parallelism with respect to their voltage thresholds. These voltage relationships are maintained throughout the range of voltages, and in general it may be seen that the magnitudes of both vasoconstriction and vasodilatation become larger as the stimulating voltage is increased.

In addition to displaying voltage dependency, the size of the sympathetic after-dilatation is also dependent, over a narrow range (1 - 30 Hz), on the frequency of stimulation. Both these correlations support the view that the changes in blood flow seen on sympathetic nerve stimulation may be attributed directly to nerve action. The frequency dependence observed for the vasoconstriction agrees with previous work in other vascular systems of the cat, particularly the work of Mellander (1960), who examined noradrenergic stimulation of resistance and capacitance vessels.

Electron microscopic evidence with regard to morphologically distinct sympathetic postganglionic fibres shows two distinct sizes

of postganglionic fibres. However, the differences were so minute as to have little physiological significance apart from the fact that electrical stimulation would not be able to achieve separation between the two sets of fibres. Furthermore, these findings do not indicate whether these two fibre populations innervate different or the same structures within the submaxillary gland.

Pharmacological attempts to separate the constrictor and dilator activities by differential adrenoceptor blockade in this study also produced negative results. It was not possible to selectively eliminate vasoconstriction so as to produce only dilatation. However, Bhoola *et al.* (1965) had succeeded in making the sympathetic dilatation appear during nerve stimulation following α-adrenergic blockade by Tolazoline. β-adrenoceptor blockade resulted only in reduction of the sympathetic after-dilatation seen in response to nerve stimulation; it could be blocked, however, by very high doses of β-adrenoceptor antagonists, the results of which are hard to interpret.

These findings must be interpreted cautiously; Bhoola *et al.* (1965) point out that large doses of α- and β-adrenoceptor antagonists block all responses to sympathetic nerve stimulation, i.e. secretion, vasoconstriction, and vasodilatation. A way of overcoming these apparently anomalous observations would entail accepting the possibility of different β-adrenoceptor subtypes in the vessels of the submaxillary gland. The possibility that more than one type of β-adrenoceptor exists cannot be totally dismissed. Levy (1966) and Moran (1966) were able to independently

show that α-methyldichloroisoprenaline and butoxamine were much more effective in blocking adrenergically-induced vasodilatation than cardiac stimulation in dogs and cats, indicating receptors with different properties.

Such a view would not be difficult to reconcile with Schachter *et al.*'s (1973) findings of enhancement of the sympathetic after-dilatation following chronic parasympathetic denervation. Although it is difficult to accept selective proliferation of a single β-adrenoceptor type following denervation, it may be surmised that denervation could serve to unmask an additional but different β-adrenoceptor subtype, which under normal conditions would only make a minor contribution to the sympathetic after-dilatation. A similar approach to explain responses to acetylcholine has been previously used by Ambache (1955), who postulated a spectrum of variation in cholinoceptor sensitivity to atropine based on receptor differences. Schachter (1967) has pointed out that in the sheep, for instance, an extreme example of this differential sensitivity exists - parasympathetic vasodilatation in the parotid gland is atropine-sensitive whereas that in the submaxillary gland is atropine-resistant, indicating different populations of cholinoceptors.

It is conceivable then, that an analogous situation could exist for the sensitivity of submaxillary gland β-adrenoceptors to noradrenaline, thereby explaining the observations seen on β-adrenoceptor blockade as well as the enhancement of the sympathetic after-dilatation after chronic parasympathetic denervation. Emmelin and

Gjörstrup (1975) have already documented the existence of a new type of β-adrenoceptor mediating secretion in the submaxillary gland of the cat. The same situation could exist in the case of the after-dilatation, in that more than one class of vascular β-adrenoceptors may be present.

Another explanation for the sympathetic after-dilatation could lie in that the increase in blood flow represents a diminishing of α-adrenoceptor-mediated vasoconstriction simultaneous with β-adrenergic vasodilatation. Although β-adrenergic dilator effects in resistance vessels result in a comparatively weak inhibition of vascular tone (Brick *et al.*, 1967; Lundvall and Järhult, 1976), such effects may be quite pronounced in the microvessels at the level of the precapillary sphincters. This suggests that the population of β-adrenoceptors in the microvasculature is more sensitive to stimulation than the α-adrenoceptor population, responding to amounts of transmitter that produce only small increases in vascular resistance. If such is the case in the vessels of the submaxillary gland of the cat, then the sequence and magnitude of blood flow changes seen after sympathetic nerve stimulation may be more readibly understandable.

Gautvik *et al.* (1974) concluded that the sympathetic after-dilatation may be due to stimulation of vascular β-adrenoceptors; however, their speculations do not consider the distribution of β-adrenoceptors in the vasculature of the gland. In this regard, it is possible to envision different populations of adrenoceptors distributed among the vessels of the submaxillary gland; depending

on the vascular bed, one type of adrenoceptor may predominate over another.

Eccles and Wilson (1974, 1975), using plethysmography, have recently examined the vasomotor responses of the blood vessels of the tongue and nasal mucosa in the cat. These blood vessels, like those of the submaxillary gland, receive sympathetic postganglionic fibres that originate in the superior cervical ganglion. The changes in blood flow seen upon nerve stimulation were similar to those seen in the submaxillary gland in response to sympathetic nerve stimulation. Vasoconstriction as well as vasodilatation were evident and could be separated by selective use of different stimulus parameters. Maximal vasodilatation was obtained using a low (1.0 - 2.0) stimulating voltage at a frequency of 25 Hz. Maximal vasoconstriction, however, was obtained with higher voltages (5.0 - 9.0) and at a lower frequency of stimulation (10 - 15 Hz).

Thus, Eccles and Wilson (1974) concluded that sympathetic vasodilator fibres from the superior cervical ganglion do innervate the nasal blood vessels. However, they speculated that these vasodilator fibres are involved with the kallikrein-kinin system, as kallikrein is found in trace amounts in the nasal mucosa (Eccles and Wilson, 1973). This interpretation may be suspect, as definitive proof of kinin-mediated vasodilatation has not yet been presented. Certainly some of their findings on the responses to nerve stimulation parallel those reached in this work as to the existence of sympathetic vasodilator fibres in the cat.

Their data is in agreement with that given in this thesis

in Fig. 5; the view that kallikrein mediates the hyperemia, however, is unacceptable. As the presence of sympathetic vasodilator fibres in the submaxillary gland of the cat may account for the sympathetic after-dilatation, there is no need to postulate that kallikrein and not vasodilator fibres mediates dilatation in the vessels of the nasal mucosa or tongue. It is more than clear from previous work (Bhoola *et al.*, 1965) and in this work that, unlike in the rabbit, the sympathetic after-dilatation in the cat cannot be accounted for by reactive hyperemia alone.

The kallikrein-kinin system can also be excluded as playing any role in the sympathetic after-dilatation. A large array of experimental evidence against the Hilton-Lewis theory has been accumulated by Schachter and his colleagues throughout the years. The most recent work (Schachter *et al.*, 1973a, b) with Bradykinin potentiating factor completely eliminates the possibility that either the chorda vasodilatation or the sympathetic after-dilatation is mediated by bradykinin. The work cited in this thesis merely serves to further confirm the latter.

The work of Emmelin *et al.*(1974) showed that myoepithelial cells in the cat submaxillary gland receive a sympathetic innervation. Sympathetic nerve stimulation results in duct pressure changes due to myoepithelial cell contraction. However, negative results were obtained in this study when testing the effects of pressure applied through the ducts on the sympathetic after-dilatation. It would appear then that myoepithelial cell contractions play little, if any,

role in mediating blood flow changes produced by nerve stimulation.

It would seem then that the origin and mechanism of the sympathetic after-dilatation remains unknown. The possibility that true sympathetic dilator fibres are present may not be ruled out on the basis of the work cited here. From previous studies (Carlson, 1907; Bhoola *et al.*, 1965; Davey *et al.*, 1965; Schachter *et al.*, 1973b), the evidence for specific dilator fibres in the cat submaxillary gland is quite strong. The possibility of different -adrenoceptors in the vessels of the submaxillary gland has already been raised; another possibility is that a different neuro-transmitter is responsible. Certainly there is recent evidence in other systems that questions the classical formulations of sympathetic and parasympathetic innervation to smooth muscle (Burnstock *et al.*, 1970; Ambache and Zar Aboo, 1971; Robinson *et al.*, 1971). Perhaps the enhancement of the sympathetic after-dilatation seen following chronic parasympathetic denervation could be due to denervation supersensitivity of a different transmitter. However, in the absence of any definitive data this remains merely speculative and will have to await future experimental work in which the subtleties and complexities of submaxillary gland function are further explored.

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Table III. Summary of Experiments[1]

Type of Experiment	Number of Animals	Number of Observations
Determination of the Relationship Between Stimulation Parameters and Evoked Vascular Response	7	~ 500
Effects of Adrenoceptor Blockade on Response to Sympath-Nerve Stimulation	4	~ 100
Enhancement of After-dilatation following Parasympathetic Decentralization	2	> 50
Electron Microscopy of Postganglionic Sympathetic Fibres	3	____
Examination of Vascular Response to Sympathetic Nerve Stimulation in the Rabbit and Effects of Adrenoceptor Stimulation and Blockade	5	~100

[1] Unless denoted otherwise, information pertains to experiments done with cats

Table I V. Quantitative Evaluation of the Vascular Response to Sympathetic Nerve Stimulation[1]

Parameter	Value[3]			
	Exp.#1	Exp.#2	Exp.#3	Exp.#4
Basal Flow Rate[2]	0.24±.08	0.56±.23	0.83±.24	0.30±.12
Maximal Extent of Vasoconstriction[2]	0.15±.09	0.36±.17	0.28±.07	0.23±.10
Decrease in Blood Flow (%)	62.54	64.29	33.73	76.66
Maximal Extent of Vasodilatation[2]	1.28±.58	1.77±1.20	1.51±.26	0.49±.18
Increase in Blood Flow (%)	221	316	146	163
Total Observations:	11	14	19	18

[1] Stimulation parameters as per text (Table I)

[2] Expressed in ml min^{-1}

[3] Mean±Standard Deviation where applicable

Table V. Regression Analysis and Least Squares Plot[1,2]

Slope[3]	Intercept[3]	Correlation Coefficient	N	Significance [4]
-0.0383	1.02503	0.0081349	17	N.S.[5]
-0.4021	1.18424	0.144327	10	N.S.
-0.1624	0.99302	0.042299	19	N.S.

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[1] The areas underneath the vasoconstriction and the after-dilatation, corresponding to the integrals of the respective flow rates, were obtained by planimetry. Within each animal these values were normalized with respect to the basal flow rate.

[2] A standard APL library reference program, Regression 2 (call number: 175 MISC), was used in this analysis; the computer used was part of the University of Alberta's MTS System.

[3] Expressed as the " best fit " approximation

[4] At the level of 0.05

[5] N.S.= not significant

Note: All stimulation parameters were kept constant except the train duration (total number of pulses). The constant parameters were as follows: voltage, 7.0 V; frequency, 15 Hz; pulse duration, 0.4 msec .

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